

CHAPTER 30

Control of *Listeria monocytogenes* by biocides in the food industry environment

Katalin Rossmoore and Cherie Drenzek

Diversey Wyandotte Corporation, Wyandotte, MI, U.S.A.

SUMMARY

The control of adventitious pathogens, such as *Listeria monocytogenes*, in the environment is a function not only of the selected biocide but also of the application involved. This paper reviews the pertinent literature and reports on the following: (1) the effect of recommended levels of some 13 biocides on several environmental surfaces contaminated with *L. monocytogenes*; (2) stainless steel coupons and ceramic tiles contaminated with milk and blood, charged with *L. monocytogenes*, and treated with 12 of the biocides; (3) control of food conveyor lubricant contaminated with milk, charged with *L. monocytogenes*, and treated with biocides; and (4) evaluation of the treatment of several process fluids with EPA-approved biocides, including glutaraldehyde, chloromethylisothiazolone, and parachlorometaxyleneol. Efficacy was affected by organic load, surface, and the chemistry of the background fluid.

INTRODUCTION

During the last six years, there have been a number of outbreaks of listeriosis, mostly associated with consumption of some dairy product contaminated with *Listeria monocytogenes*. A typical study [17] related listeriosis in dairy cows as the epidemiological source of a major outbreak. Since the dairy product was pasteurized and no fault could be found with the process, it was implied that *L. monocytogenes* might be thermotolerant to those temperatures.

More recent studies have dispelled any notions of heat resistance [5,8,9,11,14], either extra- or intracellularly. The obvious source of *L. monocytogenes* in finished dairy products is post-pasteurization contamination from the processing environment. This assumption of environmental contamination

would also be applicable to non-dairy foods, including meat and poultry products. It should be emphasized that strict adherence to environmental sanitation practices is a necessity for controlling listeriosis [28].

Listeria monocytogenes has a propensity for survival under adverse conditions, even multiplying in activated sludge in a wastewater treatment plant [22]. To mitigate the potential for survival and buildup of *L. monocytogenes*, we must focus on prevention of contamination at its source and during processing by utilizing the 'hazard analysis critical control point' (HACCP) concept [18,19]. These points include surfaces directly and indirectly in contact with product, air flow patterns (especially going from dirty to clean areas), and process fluids directly or indirectly in contact with product. In all cases just cited, the judicious use of approved chem-

ical biocides in clean areas and following good manufacturing practices (GMP) will reduce the potential for product contamination with food-borne pathogens such as *L. monocytogenes*.

AGENTS USED FOR *LISTERIA MONOCYTOGENES* CONTROL

In addition to the heat resistance studies, there is a paucity of published information on control of *L. monocytogenes*. It exhibited no unusual resistance to shortwave ultraviolet radiation; 100 MW/cm² in 4 min produced a 7-log reduction in population [30]. However, *L. monocytogenes* was more resistant than 'normal' milk microbiota to 500 ppm H₂O₂ and was apparently protected by these organisms in mixed milk culture [15].

Strikingly, *L. monocytogenes* was only one of two (the other being *Clostridium botulinum*) organisms associated with food poisoning that was susceptible to treatment with egg white lysozyme [23]. Possibly, this enzyme can be evaluated for application in certain problem areas. Of interest, especially in certain foods, is the observed synergism between NaCl content, pH, and temperature [6,12]. Higher salt levels and lower pH values require lower temperatures for *L. monocytogenes* inactivation. Neither ultra-structural [25] nor enzymatic studies [26] on the effects of disinfectants produced any unusual results with *L. monocytogenes*. Sodium hydroxide (1-2%) solubilized the cytoplasmic membrane and swelled the cell wall. Ethyl alcohol, phenol, and formaldehyde at bactericidal levels inhibited succinic and xanthine dehydrogenase but not peroxidase activity.

DISINFECTANTS AND SANITIZERS

A number of agents were evaluated for treatment of soil experimentally contaminated with *L. monocytogenes*. Contaminated soil treated with a quaternary ammonium compound at a 3% concentration applied at 0.5 L/m² was *Listeria*-free after 3 h [1]; while in another study [29], 5 l/m² of 3% formaldehyde took 5 days for soil disinfection. Differences

in protocols could not explain the variation in effectiveness between the 'quat' and formaldehyde.

A series of studies found iodine monochloride [4], hypochlorite [4,7,24], phenol, and creosote [10] effective in controlling *L. monocytogenes* in a variety of applications, including decontamination of eggs and brussel sprouts. Glutaraldehyde successfully reduced populations 3-5 logs in field and laboratory studies in dairy conveyor lubricant systems [27].

The use of biocides for controlling environmental microorganisms in the food industry is strictly controlled in the U.S. by both the EPA and the Food and Drug Administration (FDA). The FDA has the authority when there is any food contact by the biocide, whether direct or indirect. Compounds are primarily registered under the Pesticide Act of 1972 and approved for specific food industry use by the FDA. The list of sanitizers is exceedingly small and is literally limited to quaternary ammonium derivatives, halogens, and acid-based anionic detergents. Requirements for treating food industry process waters, although not as stringent as those for direct food contact, are sufficiently demanding to limit this list also [20].

Chemical species used as preservatives in food are even more limited in number for obvious reasons, and none of these are deemed adequate to handle adventitious contamination with *L. monocytogenes*. A complete discussion of this subject is beyond the scope of this study [21].

SANITIZER STUDIES

Two official sanitizer efficacy tests are applicable in the food industry: food contact surfaces [2] and non-food contact surfaces [3]. All the chemical agents mentioned in this section are described completely in Table 1. In a very extensive study, Lopes [24] compared a number of approved sanitizers against both *L. monocytogenes* and *Salmonella typhimurium*, utilizing the approved test for food contact surfaces. All compounds reduced populations a minimum of 99.999% at the recommended doses (Table 2).

Table 1
Chemical composition of selected biocides

Trivial name	Chemical name and product concentration	
Single Quat	<i>n</i> -Alkyl (C ₁₄ 50%; C ₁₂ 40%, C ₁₆ 10%) dimethyl benzyl ammonium chloride	10.0 %
Dual Quat	<i>n</i> -Alkyl (C ₁₄ 60%; C ₁₆ 30%; C ₁₂ 5%; C ₁₈ 5%) dimethyl benzyl ammonium chloride <i>n</i> -Alkyl (C ₁₂ 68%; C ₁₄ 32%) dimethyl benzyl ammonium chloride	6.25% 6.25%
		12.5%
4 Quat	<i>n</i> -Alkyl C ₁₄ 50%; C ₁₂ 40%; C ₁₆ 10%) dimethyl benzyl ammonium chloride Octyldecyl dimethyl ammonium chloride Dioctyl dimethyl ammonium chloride Didecyl dimethyl ammonium chloride	20 % 15.0 % 7.5 % 7.5 %
		50.0 %
Polymeric Quat	Poly [oxyethylene (dimethylimino) ethylene (dimethylimino) ethylene dichloride]	60 %
Dual Halogen	Sodium hypochlorite Potassium bromide	3.25% 2.00%
		5.25%
Hypochlorite	Sodium hypochlorite	5.0 %
Chlorine Dioxide	Chlorine dioxide (generated from NaClO ₂ and citric acid)	2.0 %
MCI	5-Chloro 2-methyl 4-isothiazolin 3-one 2-methyl 4-isothiazolin 3-one	1.15% 0.35%
		1.50%
PAA	Peroxyacetic acid	15 %
GLA	Glutaraldehyde	15 %
AA ₁	Dodecyl benzene sulfonic acid Orthophosphoric acid	5 % 30 %
		35 %
AA ₂	Sulfonated oleic acid Orthophosphoric acid	2.6 % 15 %
		17.6 %
PCMX	Parachlorometaxenol	1.00%

We conducted a follow-up study using the ASTM [3] method for non-food contact surfaces in which the successful endpoint is 99.9% reduction in viable population. The results are presented in Tables 3 and 4. In Table 3, two organisms are compared with *L. monocytogenes*; note that in no case is *L. monocytogenes* more resistant than *Pseudo-*

monas and *Serratia*. *Pseudomonas* is the most ubiquitous contaminant in the dairy environment; it is easy to grow and to detect. Therefore, the destruction of *Pseudomonas* by sanitizers would indicate that *L. monocytogenes* have been eliminated also.

The importance of the data presented in Table 4 relates to the role played by organic load in efficacy

Table 2

Listeria monocytogenes ATCC 13932 germicidal and detergent sanitizer test

Sanitizer type	Concentration (ppm)	Percent reduction at 30 s	Passing
Acid anionic	200	> 99.999	Yes
Single quat	100	> 99.999	Yes
Carrier bound iodine	12.5	> 99.999	Yes
Hypochlorite	100	> 99.999	Yes
Organic chlorine	100	> 99.999	Yes

Modified from Lopes [22]; see also [2].

Table 3

Comparative efficacy of non-food contact sanitizers against three organisms^a

Condition	Log ₁₀ reduction ^b									
	'Quats'				Halogens			Misc.		
	Single	Dual	Four	Poly	Hypoch	Dual	C10 ₂	MCI	GLA	PAA
ppm:	200	200	150	20	100	75	500	10	75	200
<i>Listeria</i>										
Unglazed ^c	3	3	5	4	5	3	5	5	5	5
Glazed ^c	5	5	5	4	5	4	5	5	5	5
St. Steel ^c	5	5	5	4	5	5	5	5	5	5
<i>Pseudomonas</i>										
Unglazed	3	4	2	2	5	3	5	3	5	5
Glazed	4	4	3	3	5	5	5	3	5	5
St. Steel	4	4	4	3	5	5	5	3	5	5
<i>Serratia</i>										
Unglazed	1	1	1	1	5	3	5	4	5	5
Glazed	1	1	1	1	5	5	5	4	5	5
St. Steel	1	1	1	1	5	5	5	4	5	5

^a Protocol described in [3] and [27]. ^b 3 log₁₀ reduction is passing. ^c 1" × 1" glazed and unglazed ceramic tiles and 1" × 1" 314 stainless steel coupons were used. See Table 1 for a complete description of biocides.

determinations. We must consider that in the dairy and meat packing environments, there would be no shortage of either milk or blood contamination. Surface texture is another important consideration in which differences in biocide efficacy are noted. All of the doses used were those recommended on the label, and it should be emphasized that all the products have EPA approval for this use.

The evaluation of biocides in standard laboratory tests (e.g. AOAC, ASTM) frequently fail to take

into consideration the impact of in-use environmental conditions on efficacy. We conducted both a laboratory and a field evaluation of a candidate biocide, glutaraldehyde, in a water-soluble lubricant used on dairy conveyor systems [27]. Glutaraldehyde proved most effective in the laboratory test. The results of field studies showed that 85 ppm glutaraldehyde reduced bacterial surface contamination along a dairy floor conveyor an average 4.4 log₁₀ per 60 cm² sampled. Although this study did

Table 4

Comparative efficacy of non-food contact sanitizers against *Listeria monocytogenes* in the presence of organic load^a

Condition	Log ₁₀ reduction ^b											
	'Quats'				Halogens			Misc.				
	Single	Dual	Four	Poly	Hypoch	Dual	ClO ₂	MCI	GLA	PAA	AA ₁	AA ₂
ppm:	200	200	150	20	100	75	500	10	75	200	200	200
No load												
Unglazed ^c	3	5	5	4	5	3	5	5	5	5	5	5
Glazed ^c	5	5	5	4	5	4	5	5	5	5	5	5
St. Steel ^c	5	5	5	4	5	5	5	5	5	5	5	5
1% milk												
Unglazed	3	4	3	2	4	4	5	4	5	5	4	4
Glazed	5	4	3	2	4	4	5	4	5	5	4	4
St. Steel	4	4	3	3	4	3	5	4	5	5	4	4
10% milk												
Unglazed	3	3	2	1	3	1	5	3	5	5	2	2
Glazed	3	3	2	2	3	2	5	3	5	5	2	2
St. Steel	3	3	2	1	3	2	5	4	5	5	2	2
1% Blood												
Unglazed	3	4	3	2	3	3	5	4	5	5	3	3
Glazed	5	4	3	2	3	5	5	3	5	5	3	3
St. Steel	4	4	3	2	3	3	5	5	5	5	3	3
10% Blood												
Unglazed	3	2	1	1	1	1	5	3	5	5	1	1
Glazed	3	2	2	2	1	2	5	3	5	5	1	1
St. Steel	2	2	2	1	1	2	5	5	5	5	1	1

^a Protocol described in [3] and [27]. ^b 3 log₁₀ reduction is passing. ^c 1" × 1" glazed and unglazed ceramic tiles and 1" × 1" 314 stainless steel coupons were used. See Table 1 for a complete description of biocides.

not directly involve *L. monocytogenes* for obvious reasons, earlier studies showed that detergents, such as *Pseudomonas species*, are at least as resistant as *L. monocytogenes* to this biocide.

In addition to the surfaces just mentioned, there are a number of water-based fluids involved in the dairy and meat industry subject to potential environmental contamination with *L. monocytogenes*. Before initiating a biocide evaluation study, we determined survivability of *L. monocytogenes* in four commonly encountered fluids, including two cooling water systems (sweet water and propylene glycol), brine, and conveyor lubricant (Table 5). Although there appear to be differences in survivability among the four fluids, there was no drastic lethality in any except the 35% propylene

glycol, with a three-log kill after two weeks. Sweet water, which is nothing more than potable water with added corrosion inhibitor, showed a modest one-log die-off in the same period. Brine, 23% NaCl, is commonly used in cheese and meat curing; here, after two weeks, there is only a two-log die-off. The last fluid, food-grade conveyor lubricant, differed from the other three in that it is an 'open' system continually in contact with product and environment, which add organic load and contaminants. Again, in 14 days, only a two-log drop in viability occurred.

It would appear that none of the aforementioned fluids were sufficiently inhibitory to preclude the need for biocide addition. In addition, conveyor lubricant systems could be continually contaminated

Table 5

Survival of *Listeria monocytogenes* in food process fluids

Days	23% NaCl	'Sweet water' ^a	35% Propylene glycol	1% Conveyor lubricant
0	5.5	5.8	5.6	5.4
1	4.9	5.7	5.6	4.3
2	5.0	6.1	5.3	4.3
3	4.4	5.3	5.2	4.2
4	4.4	5.4	4.5	4.2
5	4.6	5.0	3.3	4.0
6	3.8	4.9	2.4	4.0
7	3.6	ND ^b	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	ND
11	3.9	4.5	2.6	3.8
12	ND	ND	ND	ND
13	ND	ND	ND	ND
14	3.6	4.7	2.5	3.4
pH:	6.8	9.3	8.8	9.5
Temperature (°C):	22	3.5	3.5	25

^aSweet water is potable water and contains 250 ppm of a proprietary corrosion inhibitor, as does propylene glycol. ^bND = not done.

Table 6

Biocidal efficacy against *Listeria monocytogenes* in 1% food conveyor lubricant with added organic load

Biocide ^a — ppm		Log ₁₀ CFU/ml after:					
		Time zero	30 min	60 min	3 h	6 h	24 h
Control — 0	1% M ^b	6.5	6.3	6.3	5.8	6.3	6.2
	1% B ^c	6.5	6.6	6.8	6.6	6.5	6.8
GLA — 25	1% M	6.5	6.0	5.4	3.0	2.3	1.4
	1% B	6.5	6.5	6.5	6.5	6.5	6.5
	1% M	6.5	4.2	3.3	< 1	< 1	< 1
	1% B	6.5	6.3	6.5	6.3	5.0	4.8
MCI — 5	1% M	6.5	6.2	5.8	5.7	5.3	4.3
	1% B	6.5	4.7	5.8	6.5	6.4	6.5
PCMX — 500	1% M	6.5	4.8	4.4	3.7	3.5	3.1
	1% B	6.5	6.3	6.3	5.8	5.5	4.5
	1% M	6.5	4.0	3.7	3.2	2.5	< 1
	1% B	6.5	5.8	5.3	3.8	3.3	1.3

^a See Table 1 for a complete description of biocides. ^b M = whole milk. ^c B = sheep blood.

by milk and blood in field situations, contributing to the survival of resident microorganisms. We already reported on glutaraldehyde efficacy in conveyor lubricant [27] and we now report on two

other biocides, parachlorometaxylenol (PCMX), and on a commercial mixture of methylchloroiso-thiazolone and methylisothiazolone (MCI) in the presence of either 1% milk or 1% blood. The results

Table 7
Biocidal efficacy against *Listeria monocytogenes* in food process cooling waters^a

	Log ₁₀ CFU/ml after:				
	Time zero	1 h	3 h	6 h	48 h
<i>Control</i>					
Sweet water	5.8				
Propylene glycol	5.3				
<i>GLA^b — 25 ppm</i>					
Sweet water	5.8	< 1	< 1	< 1	< 1
Propylene glycol	5.3	< 1	< 1	< 1	< 1
<i>PCMX^b — 100 ppm</i>					
Sweet water	5.8	4.0	0.5	0.5	0.5
Propylene glycol	5.3	< 1	< 1	< 1	< 1
<i>MCP^b — 10 ppm</i>					
Sweet water	5.8	5.5	5.0	4.5	4
Propylene glycol	5.3	2.9	1.4	ND ^c	< 1

^a Both propylene glycol (35%, pH 9.8) and sweet water (pH 9.3) were run at 3.5°C with 1% whole milk and 250 ppm of a proprietary corrosion inhibitor. ^b See Table 1 for a complete description of biocides. ^c ND = not done.

Table 8
Efficacy of H₂O₂ against *Listeria monocytogenes*^a in 23% NaCl (brine)

% H ₂ O ₂	Log ₁₀ CFU/ml after:				
	Time zero	5 min	30 min	60 min	24 h
0	6.7	6.7	6.7	6.7	6.7
0.035	5.9	5.5	4.4	4.4	< 1
0.18	5.9	5.7	4.5	4.5	< 1
0.35	5.5	5.7	3.1	3.1	< 1

^a For culture preparation, see Rossmoore, 1988 [27].

Table 9
Survival of *Pseudomonas* and *Listeria* after drying on stainless steel in 1% whole milk

Organism ^a	Log ₁₀ CFU/ml after:				
	Time zero	30 min	60 min	3 h	24 h
<i>Pseudomonas fluorescens</i>	6.4	5.5	4.5	3.5	< 1
<i>Listeria monocytogenes</i>	6.2	5.1	4.9	3.9	2.4

^a For culture preparation, see Rossmoore, 1988 [27].

are shown in Table 6. In the presence of milk, glutaraldehyde appears to be most effective at 50 ppm, while only PCMX is effective in the presence of blood. All subsequent experiments were carried out in the presence of 1% milk. The same three biocides were evaluated in sweet water and propylene glycol and, again, PCMX and glutaraldehyde proved effective where MCI was not (Table 7).

Control of *L. monocytogenes* in brine (Table 8) is limited to very few chemical agents because of direct food contact and potential residual toxicity. The addition of H₂O₂ which gradually dissipates offers adequate control.

Two major areas of concern which we have begun to explore involve biocide efficacy against sessile populations (biofilm) and the role of airborne *Listeria* in the initiation of contamination. *Listeria* readily colonizes fittings and gaskets in experimental attachments studies (Czechowski, personal communication), and we have found (Table 9) that *L. monocytogenes* is less affected by desiccation than *Pseudomonas*. Thus, deposition on surfaces in a layer of dried milk could offer a protective additive film for survival. Such films, either dried or moist, could be expected to offer greater biocide resistance than planktonic (freely suspended) organisms, as reported in 1983 by Costerton [13].

With regard to the second point, we have presented data (Annual Meeting of the Intl. Assn. of Milk, Food, and Environmental Sanitarians, 1988) on the role of airborne organisms in the dairy on the post-pasteurization colonization and subsequent product spoilage by psychrophilic bacteria. Although we have not yet isolated *L. monocytogenes* from air samples, its isolation from many lo-

cations in the dairy, its resistance to desiccation, and the recent report on the presence of *Listeria*-like organisms in air from silage [16] suggest that we will, in time, successfully find it in air of the dairy environment.

CONCLUSIONS

We found that although efficacy differences existed among the approved biocides on which we reported, *L. monocytogenes* could be controlled by a number of these chemical agents. All agents were adversely affected by organic load in some environments. The survivability of *L. monocytogenes* in food environment processes makes the use of biocides obligatory. In general, *Pseudomonas* is more resistant to biocides than is *Listeria*. This latter fact could be important in assessing environmental efficacy, considering the differences in numbers and isolation ease in dealing with *Pseudomonas*.

Finally, it should be emphasized that prevention of contamination by strict attention to good plant hygiene programs and adherence to HACCP will go a long way in both reducing the amount of biocide needed as well as maintaining maximal efficacy.

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